# The investigation of the presence of Staphyloccous aureus in the dairy product in Karbala city

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**Abstract**

The pathogenic bacterium Staphylococcus aureus contaminated milk and milk products and causes food poisoning. Production, a variety of antibiotic resistance mechanisms, the ability to form biofilms. A total of 300 samples were collected and separated into six categories (imported milk, cow raw milk, imported dairy goods, locally produced dairy products factory, buffalo raw milk, and farm dairy products), from October 2022 to February 2023. The samples were taken at random from various cities around Karbala. S. aureus was isolated from the samples using bacteriological and biochemical techniques, 121(40.3) isolates of the 300 samples that were cultivated and identified, they were classified as S. aureus, and s.aureus were classified into MRSA and MSSA by using antibiotic susceptibility by methicillin antibiotic was found MRSA,MSSA(40.49 and 59.50) respectively. The rate of biofilm production in overall S. aureus was 100% and it was divided into three phase (strong, moderate and weak)formation the percentage of this phases among different milk and milk products was , 74(62,5%) for strong formation, 28(22,08%) for moderate formation and 19(15,42%) for weak formation.

In conclusion: an incidence of MARS with strong biofilm formation in the dairy product in Karbala province

**Keywords:** *S.aureus, biofilm formation, MRSA and dairy product.*

## INTRODUCTION

Staphylococcus aureus, and in particular MRSA, is a significant pathogen that causes severe nosocomial and community-associated infections in humans, as well as infections in economically significant livestock species. These infections can be spread through close contact with infected people or through the environment. These infections can be spread by direct contact with infected patients or through community contact (Fitzgerald, 2012).

It is possible for S. aureus to colonize a wide variety of species, such as domesticated pets, wild animals, and livestock utilized in food production (including pigs, cattle, sheep, goats, chicken, and turkey) (Cuny et al., 2015). Mastitis is an infection of the mammary tissue that affects the supply of milk in cattle in an unnatural manner. S. aureus has been known for a long time to be a primary cause of mastitis (Peton & Le Loir, 2014). On the other hand, it is known that other milk-producing ruminants, such as sheep and goats, are also susceptible to developing mastitis due to the presence of S. aureus (Achek et al., 2020).

Resistance to β-lactam group of antibiotics in S. aureus is mediated through a variety of β-lactamases or the expression of low-affinity penicillin binding protein PBP2a. The chromosomally mediated penicillin binding protein 2a initiates resistance to methicillin which confers a low affinity for all β -lactams and other unrelated group of antibiotics, thereby limiting choice for treatment ( Woodford and Livermore, 2009). β-lactamase is the predominant extracellular enzyme synthesized after exposure of S. aureus to β-lactam antibiotics (Cies et al.,2018). The enzyme is encoded in the plasmid or chromosome and its expression can either be constitutive or inductive. It deactivates the drug by cleaving the β-lactam ring. The hydrolytic ability of β-lactamase in conferring resistance in S. aureus largely depends on its location, kinetics, quantity Physiochemical conditions and interplay of determinants (Livermore, 1995).

The persistence of infection is caused by bacteria that have formed biofilm structures that shield them from the environment, antimicrobial agents, and host immune responses. These bacteria also have up to 1000-fold increased antibiotic resistance to a variety of antimicrobial agents , Antimicrobial chemotherapy now includes the treatment of biofilm-related illnesses since biofilms are resistant to therapeutic concentrations of antibiotics ( Fabres-Klein et al., 2015).

The aim of study was determine the prevalence and the contamination rate of staphylococcus aureus in raw milk , milk products and differentiation of Staphylococcus aureus MRSA from MSSA in karbala provinces as well as determination of biofilm formation.

## Material and method

1. S.aureus strain and growth condition

This study was one hundred twenty one isolated from three hundred sample of milk and milk products was classified into six groups (cow raw milk,imported milk ,imported dairy products,locally dairy products,farm dairy products and buffalo raw milk) collected from different region of Karbala province, the isolates was grown in mannitol salt agar figure(1) and incubated for 24 h at 37 C, and identified by used biochemical and microbiological method like(gram stain) instruction of Goldman and Green (2015) catalase (Collee 1996)and coagulase (Sue Katz2010).show figure(2)

2. Antimicrobial susceptibility test to differentiations between MRSA and MSSA

The possible differences in pathogenicity and virulence among strains of methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-susceptible S. aureus (MSSA) form an as yet unresolved problem. What factors contribute to virulence? On one side is the patient's ability to respond to infecting bacteria; on the other are the virulence factors produced by the bacteria, e.g., adhesins, toxins and various enzymes. The antimicrobial susceptibility profile of staphylococcus aureus isolates was performed using the disc diffusion method (Hudzicki, J. 2009).the growtu inhibition zone diameter were interpreted and ascored as

sensitive,intermediate and resistant according to the recommendation given by CLSI 2019 For the susceptibility testing, the following antimicrobial drugs (OXOID, England) and concentrations were used: b oxacillin(1mg) to classified the S.aureus to MRSA and MSSA.

3. Biofilm formation ability of S. aureus

One of S. aureus' virulence factors is its biofilm-forming capacity (Archer, 1998). Biofilm is a sessile microbial community embedded in a protective extracellular polymeric matrix, in which cells are attached to a surface or to other cells. This form

of growth displays altered physiology, gene expression and protein production, which enables S. aureus bacteria to attach to medical implants and host tissues, and underlies its resistance to therapeutic treatment (Lister & Horswill, 2014).

A method that was devised by Piechota et al. was used for the biofilm generation test, with just some minor alterations made to the method (2018). As part of the experiment, each individual strain of S. aureus was cultured on BHI

A method that was devised by Piechota et al. was used for the biofilm generation test, with just some minor alterations made to the method (2018). As part of the experiment, each individual strain of S. aureus was cultured on BHI agar that had 0.5% dextrose added to it at 37 degrees Celsius for a period of 24 hours. After the incubation period, the bacterial colony was moved to BHI broth that had been added with 0.5% dextrose. This was done in order to generate a bacterial suspension that was matched to McFarland's standard solution 0.5%, which is equivalent to 108CFU/ml. After the second round of incubation, any surplus medium was discarded before being rinsed two to three times with a standard saline solution. After transferring 200 m of the suspension into the wells of a 96-well polystyrene plate, the plate was then placed in an incubator at 37 °C for 48 hours without being shaken. After that, a fixation was performed in an oven set to 60 degrees Celsius for one hour. This was followed by the addition of 200 milliliters of crystal violate at a concentration of one percent for five minutes. After being cleaned with ordinary saline, the plate was given an hour to dry with air before being used again. In order to calculate an average of the results, each test was performed three times. After dissolving the colorant in ethanol at a concentration of 96%, the absorbency was measured with an absorbance microplate reader set to 490 nm. Whether the absorbency rate was less than 0.12 or greater than 0.42, the absorbance values were considered to be poor biofilm producers.

**Result and discussion**

Totally 121 (40.3 %) isolates were detected staphylococcus aureus and 179 (59.6%) as other bacteria .all staphylococcus aureus bacteria have ability to grow in MSA media figur(1), oxidase positive and catalase-positive and showed positive results with a slide coagulase test figure (2),

The table show prevalence of S.aureus among the group of the study(Raw cow milk, Imported milk, Imported dairy products, locally dairy products factory,farm dairy products and buffalo raw milk) table (1).

**Figure (1) growth S.aureus in MSA**

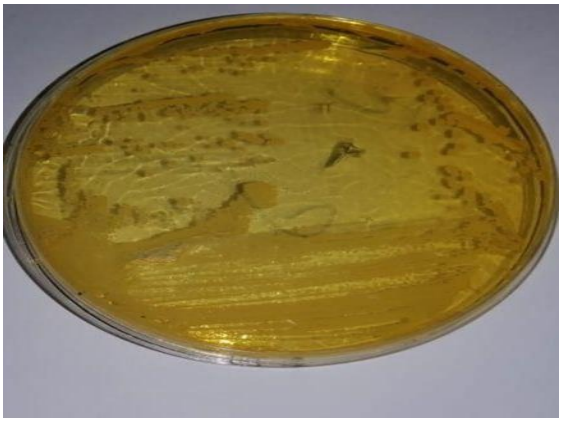
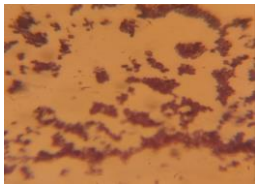
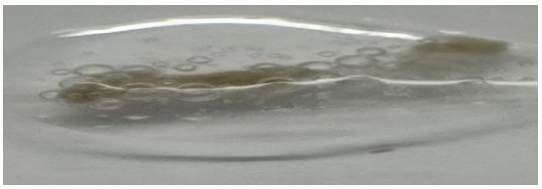


**Figure (2) the microscopic and biochemical test of S.aureus.**





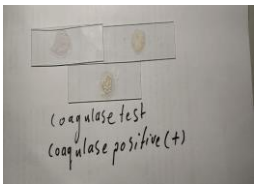



**Table (1) number and percentage of S.aureus isolation of milk and milk products**

| Group type | Number of sample | Positive sample | Percentage 0f positive sample | Negative sample |
|---|---|---|---|---|
| Cow raw milk | 50 | 17 | 34% | 33 |
| Imported milk | 50 | 13 | 26% | 37 |
| Imported dairy products | 50 | 18 | 36% | 32 |
| locally dairy products factory | 50 | 23 | 46% | 27 |
| dairy products farm | 50 | 30 | 60% | 20 |
| Buffalo raw milk | 50 | 20 | 40% | 30 |
| Total | 300 | 121 | 40,3% | 179 |

In table (1) show The high isolation rate may be due to poor personal hygiene measures during milking, transportation and display of raw milk for sale . however there are other reasons for mismanagement and the possibility of mastitis.

For persistent increase multidrug resistance S.aureus infections caused serious problem and adversely affect clinical antibiotics therapy,MRSA the strain are often resistant not only to to B-lactam antibiotics but also to antimicrobial agents commonly used in experimental hospital therapies such as aminoglycosides ,Quinolones and macrolides (Guo et al .,2020).

The antibiotic susceptibility test revealed that 49(40,49) of S. aureus isolated from milk and milk products were resistant to oxacillin (MRSA) and another 72 (59,50) of total S.aureus of study (121) was MSSA. In table (2) show the number and percentage of MRSA and MSSA in the groups of this study.

**Table (2) number and percentage of MRSA and MSSA**

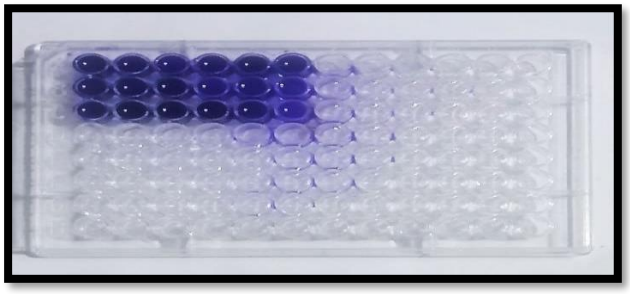
| Group type | mrsa | Percntage | mssa | percentage | number |
|---|---|---|---|---|---|
| Cow raw milk | 7 | 41,17% | 10 | 58,82% | 17 |
| Imported milk | 6 | 46,15% | 7 | 53,84% | 13 |
| Imported Dairy products | 7 | 38,88% | 11 | 61,11% | 18 |
| Locally dairy products | 9 | 39,13% | 14 | 60,86% | 23 |

| | | | | | |
|---|---|---|---|---|---|
| (factory) | | | | | |
| farm Dairy products | 12 | 40% | 18 | 60% | 30 |
| Raw buffalo milk | 8 | 40% | 12 | 60% | 20 |
| Total | 49 | 40,49 | 72 | 59,50 | 121 |

The pooled prevalence of MRSA among S. aureus isolates was 40,49% and for each type of sample as follows:7(41,17) among cow raw milk ;6(46,15%)among imported milk;7(38,88%) among imported dairy products;9(39,13%) among locally dairy products factory;:12(40%)among farm dairy products and 8(40%) from buffalo raw milk.

The capacity of S.aureus to product the biofilm help this bacteria to caused infection and caused clinical and subclinical mastitis (Dhanawade et al.,2010).in addition to, lack of sensitivity to antibioticsit is association with the formation of biofilm by S.aureus various techniques have been used to assess phenotype recognition biofilm- producing strain(Melo et al.,2013).

All 121 isolates were assayed for biofilm formation. In respect to the total ability of isolates to produce biofilm, the findings showed biofilm formation that classified to strong,modrate and weak figure(3) . The biofilm formation assay revealed that 74 (62,5) of S. aureus isolates possessed strong biofilm-formation ability, 28 (22,08 %) had moderate, and 19 (15,42 %) isolates had weak biofilm-formation ability



Determination of biofilm formation of S.aureus in 490 nm figure (3)

**Table (3) show the number and percentage of biofilm formation among different groups of the study.**

| Group type | Positive sample | Strong | % | Moderate | % | Weak | % |
|---|---|---|---|---|---|---|---|
| Cow raw milk | 17 | 11 | 64.71% | 6 | 35.29% | 0 | 0% |
| Imported milk | 13 | 9 | 69.23% | 0 | 0% | 4 | 30.77% |
| Imported dairy products | 18 | 16 | 88.89% | 2 | 11.11% | 0 | 0% |
| Locally dairy products (factory) | 23 | 12 | 52.17% | 6 | 26.09% | 5 | 21.74% |
| Farm dairy products | 30 | 18 | 60% | 6 | 20% | 6 | 20% |
| Buffalo raw milk | 20 | 8 | 40% | 8 | 40% | 4 | 20% |
| total | 121 | 74 | 62,5% | 28 | 22.08% | 19 | 15.42 % |

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